HERBAL COMPOSITION PHY-906 AND ITS USE IN CHEMOTHERAPY

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FIELD OF THE INVENTION

The present invention relates to herbal compositions and herbal extracts useful for decreasing the toxicity of drugs, including those used in the treatment of disease, especially infections and neoplasms of cancer. The methods of the present invention can be used to improve the quality of life of an individual undergoing chemotherapy. Specifically, the invention relates to the treatment of disease by increasing the therapeutic index of chemotherapy drugs by the herbal composition PHY-906. More specifically, the invention relates to the treatment of cancer by increasing the therapeutic index of cancer chemotherapy drugs by the herbal composition PHY-906.

BACKGROUND OF THE INVENTION

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

I. Herbal Medicine.

Herbal medicine has been in use for centuries by people of Asia and Europe. In the United States (US), herbs have become commercially valuable in the dietary supplement industry as well as in holistic medicine. Approximately one third of the US population has tried some form of alternative medicine at least once (Eisenberg *et al.*, 1993, N. Engl. J. Med. 328:246-252). Botanicals have also become a focal point for the identification of new active agents to treat diseases. Active compounds, derived from plant extracts, are of continuing

interest to the pharmaceutical industry. For example, taxol an antineoplastic drug obtained from the bark of the western yew tree, has been found to be useful in the treatment of breast cancer (Gomez-Espuch *et al.*, Bone Marrow Transplant (2000) 25(3):231-235).

There are many branches of herbal medicine around the world, such as Ayurveda, Unani, Sida and Traditional Chinese Medicine (TCM). While modern Western medicine typically consists of administering a single chemical entity capable of intervening a specific biochemical pathway, each formula of TCM contains hundreds of chemical entities from several herbs which are designed to interact with multiple targets in the body in a coordinated manner. Although empirical practice contributed in a significant way to the herbal composition and prescription of these ancient herbal medicines, they are also supported, to a varying degree, by a set of theories which all are distinct from that of modern Western medicine in terms of anatomy, pharmacology, pathology, diagnosis treatment, etc. Among the different herbal medicine fields, TCM has developed a more complete set of theories over several centuries which have been well documented and practiced by local physicians caring for a huge population (>1.3 billion people) in greater China and in East Asia including Korea, Japan.

II. Traditional Chinese Medicine.

Western medicine generally uses purified compounds, either natural or synthetic, mostly directed towards a single physiological target. However, the compositions used in TCM are usually composed of multiple herbs and compounds which are aimed at multiple targets in the body based on unique and holistic concepts. TCM mainly used processed crude natural products, with various combinations and formulations, to treat different conformations resulting in fewer side effects. The great potential of TCM has yet to be realized for the majority of the world's people.

The herbs in a typical TCM prescription are assigned roles as the principal herb and the secondary herbs, including assistant, adjuvant and guiding herbs. The principal herb produces

the leading effects in treating the cause or the main symptom of a disease. An assistant herb helps to strengthen the effect of the principal herb and produces leading effects in the treatment of the accompanying symptoms. There are three types of adjuvant herbs: 1) those that enhance the therapeutic effects of the principal and assistant herbs or treat tertiary symptoms, 2) those that reduce or eliminate the toxicity and other side effects of the principal and the assistant herbs and 3) those which act on complementary target tissues not specifically affected by the principal herb. A guiding herb directs the effect of other herbs to the affected site and/or coordinates and mediates the effects of the other herbs in the prescription or formulation. In contrast to most of the herbal medicines or supplements that consist of one or more parts of a single plant, the intended effects of TCM are directed at multiple tissues.

For example, a well-known TCM recipe, "Ephedra Decoction" used for treating asthma is composed of ephedra, cinnamon twig, bitter apricot kernel and licorice. Ephedra, as the principal herb, which expels cold, induces diaphoresis and facilitates the flow of the Lung Qi to relieve asthma, the main symptom. Cinnamon twig, as the assistant herb, enhances ephedra's induction of diaphoresis and warms the Channels to ensure the flow of Yang Qi for reducing headache and pantalgia. Bitter apricot kernel, as the adjuvant herb, facilitates the adverse flow of the Lung Qi and strengthens the asthma relief by ephedra. Licorice as the guiding herb moderates the effects of both ephedra and cinnamon to ensure a homeostasis of the vital Qi. While each of the four herbs clearly exhibits its respective activity, they complement as well as supplement each other when they are combined. In practice, the principal herb can be prescribed with one or more secondary herbs, depending on the symptoms at a patient's presentation (Prescriptions of Traditional Chinese Medicine, Chapter One, pp10-16, E. Zhang, editor in Chief, Publishing House, Shanghai University of Traditional Chinese Medicine, 1998).

The main theories of TCM that guide the treatment of sickness with herbal medicine and other means, such as acupuncture, are 1) the theory of Yin and Yang, 2) the theory of Five

Elements, 3) the theory of Viscera and Bowels, 4. the theory of Qi, Blood and Body Fluid, and 5) the theory of Channels and Collaterals.

In TCM, the first important aspect of making the proper diagnosis is to ascertain whether the disease is Ying or Yang. For example, those patients who have a fever, are thirsty, constipated or have a rapid pulse condition are of Yang character. Those individuals who have an aversion to cold, are not thirsty, and diarrhea and a slow pulse condition are of Yin character. The property, flavor and function of herbs can also be classified according to Ying and Yang theory. For example, herbs of cold and cool nature belong to Ying, while herbs which are warm and hot in nature belong to Yang. Herbs with sour, bitter and salty flavor belong to Ying, while herbs with pungent, sweet and bland flavor belong to Yang. Herbs with astringent and subsiding function belong to Yin, while herbs with dispersing, ascending and floating function belong to Yang. In TCM, the principles of treatment are based on the predominance or weakness of Yin and Yang. Herbs are prescribed according to their property of Ying and Yang and their function for restoring the imbalance of the Ying and Yang. In so doing, the benefit of treatment is achieved.

According to the theory of Five Elements there are five basic substances that constitute the material world (*i.e.*, wood, fire, earth, metal and water) . In TCM this theory has been used to explain the physiology and pathology of the human body and to guide clinical diagnosis and treatment. Herbal physicians have applied the laws of generation, restriction, subjugation and reverse restriction of the five elements to work out many effective and specific treatment regimens, such as reinforcing earth to generate metal (strengthening the function of the spleen to benefit the lung), replenishing water to nourish wood (nourishing the essence of the kidney to benefit the liver), supporting earth to restrict the wood (supplementing the function of the spleen to treat the hyperactivity of the liver), and strengthening water to control fire (replenishing the essence of the kidney to treat hyperactivity of the heart). Specifically, the property of some

herbs is assigned to each of the five Elements for the purposes of guiding the prescription of a TCM recipe.

In TCM, the internal organs of the human body are divided into three groups: five Viscera (the Heart, the Liver, the Spleen, the Lung and the Kidney), Six Bowels (the Gall Bladder, the Stomach, the large Intestine, the Small Intestine, the Urinary Bladder, and the Triple Warmer), the Extraordinary Organs (the Brain, the Medulla, the Bone, the Blood Vessel, the Gall Bladder, and the Uterus). In TCM, the Viscera or the Bowel are not only anatomic units, but are also concepts of physiology and pathology about interactions between different organs. For example, the heart also refers to some of the mental functions and influence functions of blood, hair, tongue and skin. Ying-Yang and the Five Elements influence the interactions among these Viscera, Bowels and Organs. The complexity of interplay of the theories is used to explain the pathology of diseases to which herbs are prescribed, as discussed below.

The prescription of herbal medicine in TCM starts with the diagnosis, which consists of four main items: interrogation, inspection, auscultation and olfaction, pulse taking and palpation. During the interrogation phase, much information is gathered, including the characteristics of the main symptoms. For instance, if the main symptom is characterized by dull pain of epigastric region, which may be relieved by warming and pressing, this suggests the insufficiency of the Spleen-Yang. Soreness and weakness of the loins and knees, intolerance of coldness with cold extremities manifests a weakness of the Kidney-Yang. During inspection, observations are made for vitality, skin color and the general appearance and the condition of the tongue. For example, a pale complexion corresponds internally to the Lung of autumn, whose Qi is dry. This may occur when Yang Qi is lacking and the circulation of Qi and blood is impeded, or when the coldness in the channels and collaterals causes them to contract.

In TCM, it is from Qi, blood and body fluid that come energy needed by the Viscera and Bowels, Channels and Collaterals, tissues and other organs for carrying-out their physiological

functions; and on which the formation and metabolism of Qi, blood and body fluid depend. Prescriptions of TCM consider the herbal effects on Qi and blood for treatments.

TCM holds that Channels, Collaterals and their subsidiary parts are distributed over the entire body It is through them that herbs exert influence on pathological targets and achieve the improvement of sickness. For example, ephedra acts on the Channels of the Lung and Urinary Bladder so as to induce sweat for relieving asthma and promoting diuresis. As noted above, clinical applications of acupuncture are also guided by the theory of Channels and Collaterals.

In summary, while the nature or property of each herb in TCM may be assigned as Yin or Yang, and to one of the Five Elements, they act through Channels and Collaterals and are mediated via Qi, Blood and Fluid to yield therapeutic effects on targets, such as Viscera and Bowels. Pathogenic factors may be disguised as decoy through the very same systems of Channels and Collaterals to adversely affect the functions of Viscera and Bowels and thus cause sickness.

III. The Patenting of Herbal Compositions in the United States.

U.S. Patents have been issued for herbal compositions used for the treatment of various diseases and other health-related problems afflicting mammals, including humans. For example, herbal compositions which include *Paeonia suffuticosa* have been found useful for treating viral infections, including infection from herpes and polio virus (U.S. Patent No. 5,411,733).

Ocular inflammation can be treated with a pharmaceutical composition containing the plant alkaloid tetrandrine (U.S. Patent No. 5,627,195). U.S. Patent No. 5,683,697 discloses a pharmaceutical composition having anti-inflammatory, anti-fever, expectorant or anti-tussive action, wherein the composition includes plant parts from the species *Melia, Angepica, Dendrobium, Impatiens, Citrus, Loranthus, Celosia, Cynanchum* and *Glehnia*. An herbal formulation comprising extracts of the roots, rhizomes, and/or vegetation of *Alphinia, Smilax, Tinospora, Tribulus, Withania* and *Zingiber* has been found to reduce or alleviate the symptoms

associated with rheumatoid arthritis, osteoarthritis, reactive arthritis and for reducing the production of proinflammatory cytokines (U.S. Patent No. 5,683,698). Compositions containing talc, silkworm excrement and the ingredients of twelve different herbs has been shown to be effective in reducing inflammation, pain and fever in mammals (U.S. Patent No. 5,908,628).

Patents have also been issued for herbal compositions which find use in the treatment of cancer and cancer-related health problems. For example, U.S. Patent No. 5,437,866 discloses a composition comprising a mixture of herbs, including species of *Scutellaria barbata*, as well as their extracts, which is used to ameliorate the effects of malignancy in humans. U.S. Patent No. 5,665,393 discloses various herbal compositions which include *Glycyrrhiza glabra L.* and *Scutellaria baicalensis Georgi*, *Rabdosia rubescens*, and *Serenoa repens* for the treatment of prostate carcinoma. Further, antitumor herbal compositions include *Astragali radix*, *Paeonia radix*, *Cinnamomi cortex*, *Rhemannia radi* and *Glycyrrhizae radix* for use in increasing antitumor activity of mitomycin D and doxorubicin (U.S. Patent No. 4,613,591 and U.S. Patent No. 4,618,495).

IV. Adverse Effects of Cancer Chemotherapy.

Medical oncology has had a great impact in changing the practice of medicine in the past several decades, as curative treatments for a variety of previously fatal malignancies have been identified. However, few categories of drugs in common use have a narrower therapeutic index and a greater potential for causing harmful side effects than do the antineoplastic drugs (Calabresi and Chabner, 1996).

Anticancer agents, like many other potent drugs with only moderate selectivity, may cause severe toxicity. Common adverse effects associated with cancer chemotherapy include, but are not limited to, gastrointestinal complications (*e.g.*, diarrhea, nausea, vomiting, anorexia and mucositis), pain, appetite loss, bone marrow/hematologic complications (*e.g.*, leukopenia, neutropenia, anemia, hemorrhage, thrombocytopenia), fatigue and sleep disturbance.

The inventors of the present invention have unexpectedly discovered that the herbal composition PHY-906 can be used in various methods for increasing the therapeutic index of one or more chemotherapeutic compounds and for modulating hematopoietic activity. The methods disclosed herein can be used to improve the quality of life for chemotherapy patients and to increase the dosage of chemotherapeutic agents because of the decreased toxicity of the agents when they are administered with PHY-906.

SUMMARY OF THE INVENTION

This invention provides the herbal composition PHY-906 combined with a pharmaceutically acceptable carrier and optionally including one or more chemotherapeutic compounds. The four plant species which are chosen to make a particular formulation of PHY-906 are each selected from one of four different groups of herbs: Scutellaria, Licorice, Peony Alba and Ziziphi Fruit. The herbs are chosen so as to obtain one or more of the desirable attributes of PHY-906, wherein such attributes include, but are not limited to, increasing the therapeutic index of one or more chemotherapeutic compounds, modulating hematopoietic activity and improving the quality of life of a mammal undergoing chemotherapy.

Chemotherapies encompassed by this invention include, but are not limited to, those useful for treating cancer, parasitic infections and microbial infections.

The compositions and methods of the present invention are useful for treating any mammal. More specifically, the methods of the present invention are useful for treating humans.

This invention further provides compositions which include a pharmaceutically acceptable carrier; material from a plant species of each of the following genera of herbs: *Scutellaria*, *Glycyrrhiza*, *Ziziphus* and *Paeonia*; and one or more chemotherapeutic compounds. More specifically this invention provides such compositions which include *Scutellaria baicalensis*, *Glycyrrhiza uralensis*, *Ziziphus jujuba*, and *Paeonia lactiflora*.

The compositions of the present invention are particularly useful with cancer chemotherapies, such as, but not limited to, treatment with an irinotecan formulation, 5-fluorouracil, VP-16 and beta-L-Dioxolane-cytidine.

The present invention also provides methods for increasing the therapeutic index of cancer therapeutic compounds used for the treatment of cancer. More specifically, the present invention provides such methods which include administering a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a plant species of each of the following genera of herbs: *Scutellaria*, *Glycyrrhiza*, *Ziziphus* and *Paeonia*. The methods of the present invention provide using material from the herbs which is in the form of a granulated extract from a concentrated aqueous liquor. Such compositions can be in an ingestible form, such as, but not limited to, powders, capsules, liquids and tablets. Alternatively, the methods of the present invention use such compositions in the form of a suppository.

The present invention also provides methods of treating diseases in mammals in need of such treatment which includes administering a therapeutically effective amount of a composition which includes a pharmaceutically acceptable carrier; material from a plant species of each of the following genera of herbs: *Scutellaria*, *Glycyrrhiza*, *Ziziphus* and *Paeonia*; and one or more chemotherapeutic compounds.

The present invention further provides methods of treating diseases in a mammal in need of such treatment which includes administering a therapeutically effective amount of one or more chemotherapeutic compounds and a composition which includes a pharmaceutically acceptable carrier; material from a plant species of each of the following genera of herbs: *Scutellaria*, *Glycyrrhiza*, *Ziziphus* and *Paeonia*. The present invention includes such methods wherein the composition is administered before the administration of the one or more chemotherapeutic compounds. The present invention also includes such methods wherein the composition is administered after the administration of the one ore more chemotherapeutic compounds.

The present invention provides methods of modulating hematopoietic activity for the treatment of a disease by administering to a mammal in need of such treatment a therapeutically effective amount of a composition consisting essentially of a pharmaceutically acceptable carrier and material from a plant species of each of the following genera of herbs: *Scutellaria*, *Glycyrrhiza*, *Ziziphus* and *Paeonia*. The present invention provides such methods wherein the material from the herbs is in the form of a granulated extract from a concentrated aqueous liquor. More specifically, the present invention provides such methods wherein the composition is in an ingestible form, such as, but not limited to, powders, capsules, liquids and tablets. Alternatively, the present invention provides such methods wherein the composition is in the form of a suppository.

The present invention also provides methods of improving the quality of life of a mammal undergoing chemotherapy which comprises administering a therapeutically effective amount of one or more chemotherapeutic compounds and a composition comprising:

i) a pharmaceutically acceptable carrier;

ii) material from a plant species of each of the following genera of herbs: *Scutellaria*, *Glycyrrhiza*, *Ziziphus* and *Paeonia*.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the effect of CPT-11 on body weight change in normal (i.e., non-tumor bearing) BDF-1 mice. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were in each group. CPT-11 was injected intra-peritoneally.

Figure 2 shows the effect of PHY-906 on weight loss in tumor bearing mice treated with CPT-11. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were injected subcutaneously with Colon 38 tumor cells. Only one dose of CPT-11 (400 mg/kg, i.p.) was injected intra-peritoneally on day zero. PHY-906 was administered orally (1 g/kg, b.i.d.) on day

zero and on a daily basis until the completion of the experiment (b.i.d. is an abbreviation for "bis in die", which means twice a day).

Figure 3 shows the effect of PHY-906 on antitumor activity of CPT-11 in Colon 38 bearing mice. Procedures were the same as described above for Figure 2. Weight of the tumors was calculated as follows (Pizzorno, 1992): Weight (mg) = (W x W x L)/2, where W(mm) is the width of the tumor and L(mm) is the length of the tumor.

Figure 4 shows the antitumor effect of L-OddC with PHY-906 on Colon 38 bearing BDF-1 mice. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were injected subcutaneously with Colon 38 tumor cells. Only one dose of L-OddC (beta-L-Dioxolane-cytidine 25 mg/kg, q.d.X5) was injected intra-peritoneally on day zero. PHY-906 was administered orally (1 g/kg, b.i.d.) on day zero and on a daily basis until the completion of the experiment (q.d. is an abreviation for "quaque die" which means once a day, q.d.X5 means each one of five mice received the dose once a day). Tumor weight was calculated as described above under Figure 3.

Figure 5 shows the antitumor effect of VP-16 with PHY-906 on Colon 38 bearing BDF-1 mice. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were injected subcutaneously with Colon 38 tumor cells. Only one dose of VP-16 (etoposide 25 mg/kg, q.d.X5) was injected intra-peritoneally on day zero. PHY-906 was administered orally (1 g/kg, b.i.d.) on day zero and on a daily basis until the completion of the experiment. Tumor weight was calculated as described above under Figure 3.

Figure 6 shows the antitumor effect of 5-fluorouracil (5-Fu) with PHY-906. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were injected subcutaneously with

Colon 38 tumor cells. Only one dose of 5-Fu (250 mg/kg) was injected intra-peritoneally on day zero. PHY-906 was administered orally (1 g/kg, b.i.d.) on day zero and on a daily basis until the completion of the experiment. Tumor weight was calculated as described above under Figure 3.

Figure 7 shows the antitumor effect of 5-fluorouracil (5-Fu) with PHY-906. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were injected subcutaneously with Colon 38 tumor cells. 5-Fu (30 mg/kg, q.d.x5) was injected intra-peritoneally daily. PHY-906 was administered orally (1 g/kg, b.i.d.) on day zero and on a daily basis until the completion of the experiment. Tumor weight was calculated as described above under Figure 3.

Figure 8 shows the antitumor effect of CPT-11 with PHY-906 versus Loperamide on Colon 38 bearing BDF-1 mice. Five female BDF-1 mice (8-10 weeks old, average weight about 20g) were injected subcutaneously with Colon 38 tumor cells. Mice either received no treatment, PHY-906 alone, CPT-11 alone, CPT-11 and PHY-906, or Loperamide alone. The PHY-906 and CPT-11 were administered as set forth in Figure 3. Only one dose of Loperamide was injected peritoneally (2 mg/kg, po, b.i.d.)on day zero. Tumor weight was calculated as described above under Figure 3.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Chemotherapy.

In general, chemotherapy refers to the treatment of disease, especially neoplasms, parasitic infections and microbial diseases, with chemical agents that in some manner act on the infective organisms or tumors.

A. Cancer Chemotherapy.

Introduction. Drugs for treating cancer include the more conventional natural products such as paclitaxel (TAXOL), the semisynthetics such as etoposide, and many newer, diverse

agents such as interleukin-2 and all-trans-retinoic acid. For a comprehensive list of chemotherapeutic agents useful in treating neoplastic diseases, see, for example, Table X-1 at pages 1227-1229 of Calabresi and Chabner (1996).

The major adverse effects associated with commonly administered cancer chemotherapies are provided in Table 1.

Table 1. Major adverse effects of cancer chemotherapy.

Major Adverse Health Effects	Antineoplastic Agent
Pancreatitis	VP-16, ara C
Alopecia	VP-16, Doxorubicin, Taxol, 5-FU, araC
Cardiotoxicity	Taxol, Doxorubicin
Cutaneous	Doxorubicin
Diarrhea	CPT-11
Dyspnea	ara C
Flush	Tamoxifen
Fever/Chills	VP-16, Doxrubicin
Hepatotoxicity	VP, Taxol, ara C, Methotrexate
Nephrotoxicity	Cisplatin
Ototoxicity	Cisplatin
Bone Marrow Hypoplasia	Almost all anticancer drugs

5-fluorouracil. The fluoropyimidine, 5-fluorouracil (5-FU), exhibits a broad spectrum of clinical activity. It remains one of the most active agents in the treatment of colorectal cancer both in the adjuvant and advanced disease setting, and in other GI malignancies as well (Pinedo and Peters, 1988). In addition, this agent is active against cancers of the breast, and head and neck.

Recent advances in the therapy of colorectal cancer have used biochemical modulation to selectively activate specific pyrimidine metabolic pathways. The reduced folate, leucovorin (LV), is an effective biochemical modulator and has been used in combination with 5-FU treatment (Peters and Van Groeningen, 1991; Joulia, et al., 1999).

The response rate to 5-FU in patients with advanced disease is improved from 10% -12% (5-FU treatment alone) to 20%-30% (5-FU/LV treatment).

For a detailed description of the therapeutic uses of the fluoropyrimidine analogs, including 5-FU, see, for example, Chabner *et al.*, 1996.

CPT-11. Irinotecan (CPT-11) is a semi-synthetic camptothecin analogue that inhibits topoisomerase I in the replicating cell. It exhibits anti-tumor activity in cancer patients who fail first-line treatment with 5-FU/LV (Bleiberg, 1999; Stucky-Marshall, 1999).

While CPT-11 is FDA-approved as a second-line therapy for patients with advanced colorectal cancer, the observed response rates are on the order of only 10% -15%.

The main side effects associated with this agent include leukopenia, anemia, nausea/vomiting, anorexia, and diarrhea. It is, therefore, desirable to develop a modulator agent that can either enhance the efficacy of the anti-tumor activity of CPT-11 and/or alleviate some of the toxic side effects associated with CPT-11 treatment so that the overall quality of life and performance status of the cancer patient is improved.

VP-16 (etoposide). VP-16, also known as etoposide, shows significant clinical activity against small-cell lung cancer, testicular cancer, lymphoma and leukemia (O'Dwyer, P., et al., Etoposide (VP-16-213), Current Status of an Active Anti-cancer Drug, New Engl. J. Med. 312:692-700 (1985)) and include neoplasms seen in Hodgkin's disease, Papillomavirus and diffuse histiocytic lymphoma.

It is believed that etoposide blocks the catalytic activity of DNA topoisomerase II by stabilizing an enzyme-DNA complex in which the DNA is cleaved and covalently linked to the enzyme. See Chen, G. L., Yang, L., Rowe T. C., Halligan, B. D., Tewey, K., and Liu, L., J.

Biol. Chem., 259:13560 (1984); Ross, W., Rowe, T., Glisson, B., Yalowich, J., and Liu, L., Cancer Res., 44:5857 (1984); Rowe, T., Kuppfer, G., and Ross, W., Biochem. Pharmacol., 34:2483 (1985), which are all herein specifically incorporated by reference.

By way of background, topoisomerases are enzymes which control the topological state of DNA. Type II topoisomerases catalyze DNA strand passage through transient double strand breaks in the DNA. The resulting change in the linking number of DNA allows these enzymes to mediate DNA interconversions, such as supercoiling and relaxation of supercoiling, catenation and decatenation, knotting, and unknotting. See Wang, J. C., Annu. Rev. Biochem., 54:665 (1985) and Maxwell, A., and Gellert, M., Adv. Protein Chem., 38:69 (1986), which are herein specifically incorporated by reference.

Type II DNA topoisomerase enzymes have been shown to be involved in a number of vital cellular processes, including DNA replication and transcription, and chromosomal segregation. These enzymes, therefore, are a critical target for the action of a wide variety of anticancer drugs, including etoposide and teniposide. The key step leading to cell death may be the capability of these drugs to block the catalytic activity of DNA topoisomerase II, as noted above.

OddC (beta-L-dioxolane-cytidine). Beta-L-dioxolane-cytidine [(-)-OddC] is the first nucleoside analogue with the unnatural L configuration shown to have anticancer activity. (Grove et al., Cancer Res (1996) 56(18):4187-4191). This compound has been shown to have a potent antitumor activity in human prostate and hepatocellular xenograft tumor models (Grove et al., Cancer Res (1995) 55:3008-3011). Further, OddC has been shown to be effective against hyperproliferative activity in human keratinocytes in vitro (Schwartz et al., Skin Pharmacol Appl Skin Physiol (1998) 11(4-5):207-213).

This compound works by rapid translocation into cells by both equilibrative-sensitive and -insensitive nucleoside transport systems where it is incorporated into DNA of cells. DNA

incorporation leads to degradation of DNA into large fragments without generation of internucleosomal laddering.

Quality of Life. Standard evaluation measures for the success of cancer treatments include, but are not limited to, changes in tumor mass and type as well as the rate and amount of tumor spreading (both locally to and distant to the tumor(s) being evaluated). One skilled in the art of chemotherapy evaluations can also determine whether a particular treatment appears to enhance a patient's life expectancy and quality of life (even for those patients not responding to the usual treatments). For example, effective treatment of gastrointestinal diseases may be determined by several criteria, including, but not limited to, an enteritis score (based upon a composite score of clinical symptoms such as abdominal pain, cramping, stool guaiac and diarrhea), as well as related endpoints such as percent chemotherapy dose delivered, days of hospitalization, transfusions, intravenous fluid therapy, antimotility agents, and ability to eat.

With respect to a treatment effect, the subjective symptoms of the patient do not always coincide with the result of the test conducted by the doctor. For example, even in the case where an unfavorable test result is obtained, when the occurrence of urinary incontinence and voiding are reduced, the patient believes the treatment has worked, with the result that the quality of life (QOL) is improved. During chemotherapy the negative side-effects in the life of the patients, such as hair loss, reduction in weight, loss of appetite, fatigue, diarrhea, nausea, vomiting, etc. can be persistent and result in chronic torment, night and day, that can be unbearable to the patients, both physically and mentally. Thus, therapeutic effectiveness of methods of the present invention is meant to refer not only to partial or entire relief from the pain or reduction in tumor growth or cancer regression, but relief as a consequence of reduced or eliminated side-effects traditionally associated with treatment, with the overall result being an enhanced quality of life.

Baseline evaluations can be entered as part of the treatment protocol whereby various criteria are measured and correlated with QOL. Further, patients can report on a patient diary events such as feeling "fair" or experiencing "moderate" pain. These measures are then used

during and after treatment to evaluate whether the patient feels that the quality of life has improved.

B. Chemotherapy of Parasitic Infections.

Parasitic protozoa are responsible for a wide variety of infections in man and animals and many diseases caused by parasitic protozoa are life threatening to the host. For example, malaria remains a significant health threat to humans despite massive international attempts to eradicate the disease; trypanosomiasis such as Chagas disease caused by *Trypanosoma cruzi* and African sleeping sickness caused by *T. brucei* are not uncommon in Africa and South America; and opportunistic infections in immunocompromised hosts caused by *Pneumocystis carinii*, *Toxoplasma gondii*, *Cryptosporidium* spp. are becoming increasingly significant in the developed countries.

In some protozoal diseases, such as Chagas disease, there is no satisfactory treatment; in others, drug-resistant strains of the protozoa may develop. Accordingly, there exists a continued need to identify new and effective anti-protozoal drugs. However, antiparasitic drug discovery has been, for the most part, a random and laborious process through biological screening of natural products and synthetic compounds against a panel of parasites.

Despite encouraging progress in vaccine development, chemotherapy remains the single most effective, efficient, and inexpensive means to control most parasitic infections (Tracy and Webster (1996)). Drugs available now are especially effective in treating human infections caused by flukes and intestinal parasites. But new or better pharmaceuticals are urgently required, both to combat such systemic infections as cysticercosis, filariasis, leishmaniasis, trichinosis, and trypanosomiasis and to counteract development of drug resistance manifested especially by malaria and other protozoan parasites. Protozoan parasites develop resistance to drugs far more readily than do helminths, consistent with their more rapid proliferation in the host.

It is essential that antiparasitic drugs be safe and effective in patients. The therapeutic efficacy of antiparasitic drugs are complex and are dependent upon the host, the parasite, and the environmental factors. Thus, the best drugs and optimal dose regimens are often determined by trial and error rather than from careful pharmacokinetics and pharmacodynamic studies of patients with endemic infections. For proper evaluation, population-based chemotherapy should be instituted only after appropriate epidemiological studies divulge patterns of transmission and the relationship of age-specific prevalence and intensity of infection to disease. For optimal results, chemotherapy should be combined with other public health measures appropriate for the particular infection, environment and host population. The ideal agent for mass chemotherapy would have a broad spectrum of activity against all developmental stages of infecting parasites. It also would be safe at a high therapeutic doses taken orally for one day only; be chemically stable under conditions of use; be effective as an inducer of drug resistance; and be inexpensive. At the present, few available antiparasitic drugs meet these criteria.

Chemotherapeutic agents that are effective against asexual erythrocytic malarial parasites include chloroquine, quinine, quinidine, mefloquine, and halofantrine. Other drugs such as pyrimethamine, sulfonamides, sulfones, and tetracyclines, are slower acting and less effective than the above agents, and therefore are usually used in combination with other chemotherapeutic agents. Agents such as atovaquone, chloroquine, diloxanide furoate, eflornithine, emetine and dehydroemetine, 8-hydroxyquinolines, melarsoprol, metronidazole, nifurtimox, pentamidine, quinacrine, sodium stibogluconate, and suramin are effective in treating parasitic infections including trypanosomiasis, leishmaniasis, amebiasis, giardiasis, and trichomoniasis. Lastly, infections with parasitic worms, helminthiasis, are usually treated with anthelmintic drugs such as benzimidazole, diethylcarbamazine, ivermectin, metrifonate, niclosamide, oxamniquine, piperazine, praziquantel, and pyrantel pamoate. For a review of drugs for chemotherapy of parasitic infections, see,.Tracy and Webster, Id.

C. Chemotherapy of Microbial Diseases.

In 1936, favorable clinical results using sulfanilamide in puerperal sepsis and meningococcal infections reported by Colebrook and Kenny and Buttle and coworkers awakened the medical profession to the new field of antibacterial chemotherapy. In 1941, penicillin was mass produced and first made available for limited clinical trial. Presently, at least 30% of all hospitalized patients receive one or more courses of therapy with antibiotics, and millions of potentially fatal infections have been cured.

Antibiotics are substances produced by various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of other microorganisms and eventually may destroy them. However, common usage extends the term to include synthetic bacterial agents, such as sulfonamides and quinolones, which are not products of microbes. Antibiotics differ in physical, chemical, and pharmacological properties; antibacterial spectra; and mechanisms of action.

The most common classification of antimicrobial agents which is based on chemical structure and proposed mechanism of action is the following: (1) agents that inhibit synthesis of bacterial cell walls; for example, the penicillins and cephalospoins, which are structurally similar, and dissimilar agents such as cycloserine, vancomycin, bacitracin, and the *imidazole* antifungal agents such as miconazole, ketoconazole, and clotrimazole; (2) agents that act directly on the cell membrane of the microorganism, affecting permeability and leading to leakage of intracellular compounds; these include the detergents, polymyxin and colistimethate, and the polyene antifungal agents,, that bind to cell-wall sterols; (3) agents that affect the function of 30 S or %0 S ribosomal subunits to cause a reversible inhibition of protein synthesis; these bacteriostatic drugs include chloramphenicol, the tetracyclines, erythromycin, and clindamycin; (4) agents that bind to the 30 S ribosomal subunit and alter protein synthesis, which eventually leads to cell death; these include the aminoglycosides; (5) agents that affect nucleic acid metabolism, such as the rifamycins (*e.g.*, rifampin), which inhibit DNA-dependent RNA

polymerase, and the quinolones, which inhibit gyrase; (6) the antimetabolites, including trimethoprim and the sulfonamides, which block specific metabolic steps that are essential to microorganisms; (7) nucleic acid analogs, such as zidovudine, ganciclovir, vidarabine, and acyclovir, which inhibit viral enzymes that are essential for DNA synthesis, thus halting viral replication. (See,.Tracy and Webster, page 1029.)

Whether an antibiotic is effective in treating an infection depends on several factors. In order for an antibiotic to be effective, a sufficient concentration of the antibiotic must be achieved at the site of infection to inhibit bacterial growth. However, the concentration of the drug must remain below those that are toxic to human cells. If the concentration of antibiotic required to inhibit or kill the microorganism is greater than the concentration that can be safely achieved, the microorganism is considered to be resistant to the antibiotic. Bacteria can be resistant to an antimicrobial agent because the agent fails to reach its target, the agent is inactivated, or the target is altered. Some bacteria produce enzymes that reside at or within the cell surface and inactivate the drug. Others possess impermeable cell membranes that prevent the influx of the drug. Some bacteria are deficient in aqueous channels made up of porins that hydrophilic agents use to traverse the outer membrane of bacteria, while others lack the transport system that is required for entrance of the drug into the bacterial cell. The emergence of antibiotic resistant pathogens has led to an ever-increasing need for new drugs and new methods of treating antimicrobial diseases.

II. PHY-906.

Introduction. PHY-906 is a traditional Chinese botanical formulation composed of four herbs each of which is selected from one of four herb groups. The four herb groups are commonly known as Scutellaria, sometimes known as Scutelleria; Licorice, Peony Alba and Ziziphi Fruit (Table 2). Thus, one plant species is chosen from each one of the four plant groups provided in Table 2 in order to produce the desired herbal compositions of the present invention.

While particular combinations of the listed plant species are provided as examples of preferred PHY-906 formulations, the compositions and methods of this invention encompass any combination of four plant species wherein a plant species is selected from each one of the four groups in Table 2. This invention encompasses any such combination of such herbs which have at least one of the biological activities or desired effects as described to PHY-906 as described herein.

Table 2. Examples of particular species of four genera which can be used to make PHY-906.

Common English Name of TCM Herbal Group			
Scutellaria	**Licorice**	**Peony Alba**	**Ziziphi Fruit**
Anemone rivularis Buch.-Ham. ex DC.	*Abrus mollis* Hance	*Paeonia delavayi* Franch. var. lutea (Delavay ex Franch.) Finet et Gagnep.	*Ziziphus jujuba* Mill.
Thalictrum omelense W.T. Wang et S.H. Wang	*Glycyrrhiza aspera* Pall.	*Paeonia lactiflora* Pall.	*Ziziphus jujuba* Mill. var. inermis
Mahonia bealei (Fort.) Carr.	*Glycyrrhiza eurycarpa* P.C.Li	*Paeonia mairei* Levi.	
Nandina domestica Thunb.	*Glycyrrhiza glabra* L.	*Paeonia obovata* Maxim. var. willmottiae (Stapi) Stern	
Scutellaria amoena C.H. Wright	*Glycyrrhiza inflata* Bat.	*Daphne papyracea* Wall. ex Steud.	

Scutellaria	Licorice	Peony Alba	Ziziphi Fruit
Scutellaria amoena C.H. Wright var. cinerea Hand.-Mazz.	*Glycyrrhiza squamulosa* Franch.	*Cynanchum otophyllum* Schneid.	
Scutellaria baicalensis Georgi	*Glycyrrhiza uralensis* Fisch.	*Codonopsis lanceolara* Sieb. et Zucc. Trautv.	
Scutellaria baicalensis Georgi var. albiflora K. Onuma	*Phlomis betonicoides* Diels		
Scutellaria chungtienensis C.Y. Wu			
Scutellaria hypericifolia Levl			
Scutellaria likiangensia Diels			
Scutellaria obtusifolia Hemsl. var. trinervata (Vant.) C.Y. Wu et H.W. Li			
Scutellaria regeliana Nakai			

Scutellaria	Licorice	Peony Alba	Ziziphi Fruit
Scutellaria regeliana Nakai var. ikonnikovii (Juz.) C.Y. Wu et H.W. Li			
Scutellaria rehderiana Diels			
Scutellaria tenax W.W. Smith var. patentipilosa (Hand. -Marz.) C.Y. Wu			
Scutellaria viscidula Bunge			

This herbal formula has been long used in Asia to treat a variety of ailments such as cardiac distention, abdominal spasms, fever, headache, vomiting, retching, thirst and mucous-like stool (Hani Oka and Taki No, 1998).

A preferred formulation of PHY-906 is provided in Table 3.

Table 3. Herbal Ingredients of TCM Formula PHY-906

Scientific	Percentage	Common Name	Traditional Use
Scutellaria baicalensis	33.3	Scute Baical Skullcap Root	Used to reduce capillary permeability; to reduce inflammation: to treat enteritis and dysentery; increase the secretion of bile to treat jaundice; to relieve muscle spasms to treat coughing; to expel parasites.
Glycyrrhiza uralensis	22.2	Licorice Root	Used to moisten the lungs and stop coughs; to relax spasm and stop pain; to moderate the action of herbs: to reduce fire and release toxins.
Ziziphus jujuba	22.2	Date	Has diuretic and strengthening effects
Paeonia lactiflora	22.2	White Peony Root	Used to suppress and soothe pain: to soothe ligaments and purify the blood

An alternative formulation of PHY-906 has the herbs *Scutellaria, Glycyrrhiza, Ziziphus,* and *Paeonia* in the following relative proportions: 4/14:3/14:4/14:3/14, respectively.

While specific ratios of the herbs of PHY-906 are provided as examples, the compositions and methods of this invention encompass any ratios of the four herbal components which have the desired biological activity as described herein.

Currently, both gelatin capsules and granule pouches of PHY-906 are produced by Sun Ten Laboratories, Inc. in Irvine, CA (a sister company of Sun Ten Pharmaceutical Co. Ltd. in Taiwan) using the formulation provided in Table 3. This formulation of PHY-906 has been distributed and sold as a dietary supplement since 1983 by Brion Herbs Corporation (12020 B Centralia Road, Hawaiian Garden, CA, 90716).

Production. A brief review of a process which can be used for producing PHY-906 is provided. First, the proper ratios of the ingredients of the herbal raw materials are placed in a jacketed reactor and extracted with water at a elevated constant temperature with mixing. The ratios are set forth in the Manufacturing Instruction reproduced from Master Formula Record.

The solid materials are then separated from the liquid with a 120-mesh screen. The filtrate is collected and then concentrated by evaporating the water under reduced pressure. The concentrated liquor is spray dried at an elevated temperature to yield dry powder which is then processed to yield granulated powder. This bulk substance is then formulated into the desired dosage form.

Process controls are utilized to ensure the uniformity and integrity of the product. Such process controls include, but are not limited to, checking the volume of the process liquor, HPLC determinations to establish Chemical Fingerprintings to verify identity of the raw materials, inspections and tests of intermediate and final products. Accepted Quality Level (AQL) Limits are established for each conducted analysis and for each step of the manufacturing and control of production.

All of the components used in the production process are assigned a specific lot number in the Production Instruction Record. Quality control records are reviewed before a batch is released.

Purified marker substances are used for identification and quality control of the raw materials as well as the herbal substances. Table 4 lists the marker substances of each raw material used in preparation of PHY-906 herbal substance.

Table 4. Marker Substances for Herbal Ingredients of PHY-906

Herb	Origin of Herb Producing Place	Marker Substance
Scutellaria baicalensis	Shang Xi Province, China	Baicalin
Glycyrrhiza uralensis Fisch.	Inner Mongolia, China	Glycyrrhizin
Ziziphus jujuba Mill.	Hebei/Shangtong Province,	Chelidonic Acid
Paeonia lactiflora Pall.	An Hwei Province, China	Paeoniflorin

III. Pharmaceutical Formulations.

The compositions of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

The present invention further provides compositions containing one or more agents which treat various types of cancer and/or modulate hematopoietic activity, such as the immodulation of tuberculosis (T.B.), natural killer cells (NK), monocytes and dendritic cells.

While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action.

PHY-906 can be used in the form of a medicinal preparation, for example, in solid, semi-solid or liquid form which contains PHY-906, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for external, enteral or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. Formulations of the present invention encompass those which include talc, water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, corn starch, keratin, colloidal silica, potato starch, urea and other carriers suitable for use in manufacturing preparations, in solid, semisolid or liquid form and in addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used.

For preparing solid compositions such as tablets or capsules, PHY-906 is mixed with a pharmaceutical carrier (*e.g.,* conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums) and other pharmaceutical diluents (*e.g.*, water) to form a solid preformulation composition containing a substantially homogeneous mixture of PHY-906, or a non-toxic pharmaceutically acceptable salt thereof. When referring to the preformulation compositions as substantially homogenous, it is meant that the active ingredients are dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage

forms of the type described above containing an effective amount of the composition of the present invention, preferably in capsules.

The tablets or pills containing PHY-906 can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

The liquid forms, in which PHY-906 may be incorporated for administration orally or by injection, include aqueous solution, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil as well as elixirs and similar pharmaceutical vehicles. Suitable dispersing or suspending agents for aqueous suspensions include synthetic natural gums, such as tragacanth, acacia, alginate, dextran, sodium carboxymethyl cellulose, methylcellulose, polyvinylpyrrolidone or gelatin.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for reconstitution with water or other suitable vehicles before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); preservatives (*e.g.*, methyl or propyl p-hydroxybenzoates or sorbic acid); and artificial or natural colors and/or sweeteners.

For buccal administration, the compositions of the present invention may take the form of tablets or lozenges formulated in conventional manners.

PHY-906 may also be formulated for parenteral administration by injection, which includes using conventional catheterization techniques or infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules, or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredients may be in powder form for reconstitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

In practicing the methods of this invention, PHY-906 may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for cancer chemotherapy according to generally accepted medical practice.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

Actual methods for preparing administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pa. (1980), which is incorporated herein by reference.

"Therapeutic index" is used to designate a qualitative statement of the selectivity of a drug when a therapeutic and an untoward effect are being compared. For example, if the untoward effect is designated as *T* (for toxic) and the therapeutic effect as *E*, the therapeutic index may be defined as TD50/ED50 or a similar ratio at some other arbitrary levels of response.

EXAMPLES

Example 1. Herbal Preparation.

PHY-906 was provided by SunTen Pharmaceutical Ltd. from Taiwan using the formulation set forth in Table 3. All of the herbal extract were freshly prepared prior to the studies. PHY-906 (1g) was extracted with 10 ml of 80°C H_2O and incubated at 80°C for 30 min. The supernatant was separated from the debris by centrifugation (2060g, 15 min). The concentration of supernatant was considered as 100 mg/ml, based on the dry weight of herb. T

he supernatant was passed through a 0.45 μm sterile Acrodisc filter (Gelman Sciences) prior to examination for growth inhibition in tissue culture.

Example 2. Evaluation of Toxicity of PHY-906 on Different Cell Lines.

Briefly, one gram of each batch of PHY-906 was added with 10 ml of water (1 mg/ml). See Table 5 for the batch properties.

Table 5. Batch Properties PHY-906

Property	Batch A	Batch B
Origin	Taiwan, Sun-Ten	Taiwan, Sun-Ten
Preparation method	Standard	Standard

The supernatant was collected after centrifugation and filtered through a 0.22 μm filter. Four cell types were used to test for biological effects of each batch of PHY-906: a) KB cells (ATCC cat. # CCL-17); b) HepG2 cells (ATCC cat # HB-8065); c) T-cell lymphoma cell line (CEM cells); d) Colon 38 and e) HCT116 (ATCC cat # CCL-247).

The carcinoma cells ($1x10^4$) were seeded into a 24-well plate in either 1 ml of MEME medium or RPMI-1640 with 10% FBS and 100 μg Karamycin on day 0. The PHY-906 extract was added to the cells at log concentrations and incubated at 37°C for 3 days on day 1. The medium was then removed and the cells stained with 0.3 ml of 0.5% (w/v) methylene blue solution (in 50% EtOH) for 30 min. The plates were washed 3 times with tap water, dried and the cell layer was lysed with 1 ml of 1% Sarkosyl solution (in PBS). The lysate solution was read on a Elx800 kinetic microplate reader (Bio-Tec Instruments, Inc.) at 595 nm.

Cytotoxicity study of human T-cell lymphoma cell line (CEM) CEM cells ($5x10^4$) were grown in 1 ml RPMI 1640 medium with 20% displayed fetal bovine serum. The PHY-906 extract was added at day 0. The growth of cells was accessed 3 days post addition of PHY-906. The number of cells were estimated as hemacytometer.

The results of the assays using the two (2) batches are displayed in Table 6. Based on these data, PHY-906 sources A and B have relatively for KB, HEP2G and HCT116 cells, while having significantly greater cytotoxic effects against Colon 38 cells (see Table 6).

Table 6. Cytotoxicity of Traditional Herbal Formulations in Different Cell Lines.

	IC_{50} (mg/ml)[a]				
Herbal Formulation	KB	HepG2	CEM	Colon 38	HCT116
PHY-906A	1.35 ± 0.52	0.28 ± 0.17	1.45 ± 0.45	0.08	1.3
PHY906B	1.80 ± 0.99	0.17 ± 0.12	1.28 ± 0.02	0.08	1.2

[a] Based on the dry weight of herbal formulation.

Example 3. Body Weights of Non-tumor Bearing Mice Treated With CPT-11.

To determine the maximum tolerable dose of CPT-11 for mice, the effect of CPT-11 on weight loss in normal (*i.e.*, tumor-free), female BDF-1 mice was studied using five different dosages: 100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg, 600 mg/kg and 800 mg/kg of animal body weight. Mice which received no CPT-11 treatments acted as controls.

One dose of CPT-11 was administered intra-peritoneally to each mouse at the beginning of the study, and the weight loss of the animal was then monitored daily for 12 days. Both the duration and extent of the weight loss are sensitive to the dose of CPT-11 administered to the animal.

Dosages lower than 200 mg/kg showed little effect on body weight compared to mice that received no treatment with CPT-11 (data not provided). In contrast, drug dosages higher than 600 mg/kg resulted in animal death on the second day after CPT-11 administration (data not provided).

The average body weights of the mice treated with 300 mg/kg were significantly less than those of the mice that received no treatment with CPT-11 until 5 days after treatment (Figure 1). The average body weights of these two groups of mice were not significantly different from 5 days after treatment until the end of the trial. In contrast, weight loss was observed immediately after 400 mg/kg CPT-11 treatment and continued for six days. The animals gradually started to recover from toxicity on day twelve but their average body weights remained significantly less

than those that received no treatment throughout the twelve day trial. Thus, the mice were able to tolerate up to 400 mg/kg of dosage.

Example 4. Body Weights of Colon 38 Inoculated Mice Treated with CPT-11 and PHY-906.

PHY-906 was evaluated as a modulator of CPT-11 therapy for toxic side effects in mice inoculated with Colon 38 tumor cells. Based on the findings as detailed in Example 3, we selected a dose of 400 mg/kg for treatments with CPT-11.

Mice were subjected to a subcutaneous injection of murine Colon 38 tumor cells. Seven days after inoculation with the cancer cells, mice were treated with CPT-11 (400 mg/kg) intra-peritoneally and oral administration of PHY-906 (1 g/kg, b.i.d.). The animals were then administered only with the same dose of PHY-906 continuously for the rest of the experiment. Control mice were inoculated with the tumor cells but did not receive either CPT-11 or PHY-906 treatment.

Figure 2 shows the comparison of the body weights of CPT-11 treated mice both with and without PHY-906 treatment. The results shows that the CPT-11 treated animals receiving supplemental treatment with PHY-906 at 1 g/kg suffered a significantly lower degree of body weight loss (see Table 7 for a summary of the statistical results). In addition, a shorter length of time was required for the mice treated with CPT-11/PHY-906 at 1 g/kg to return to their original body weights.

Table 7. Statistical Analysis of PHY-906 on Weight Loss in Tumor Bearing Mice Treated With CPT-11.

N=5	No Treatment	CPT-11	CPT-11/PHY-906 1 g/kg	CPT-11/PHY-906 0.5 g/kg	CPT-11/PHY-906 0.25 g/kg
No Treatment	----	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.01$
CPT-11	$P < 0.01$	----	$P < 0.01$	$P > 0.1$	$P > 0.1$
CPT-11/PHY-906 1 g/kg	$P < 0.05$	$P < 0.01$	----	$P < 0.01$	$P < 0.01$
CPT-11/PHY-906 0.5 g/kg	$P < 0.01$	$P > 0.1$	$P < 0.01$	----	$P > 0.1$
CPT-11/PHY-906 0.25 g/kg	$P < 0.01$	$P > 0.1$	$P < 0.01$	$P > 0.1$	----

Example 5. Tumor Weight of Colon 38 Inoculated Mice Treated with CPT-11 and PHY-906.

Mice were treated as set forth in Example 4 and evaluated for tumor weights over a nine day period.

The results demonstrate that treatment with PHY-906 neither impedes nor impairs the antitumor efficacy of the CPT-11 (Figure 3). In fact, the data suggest that this herbal medicine may actually enhance CPT-11 anti-tumor activity.

These preliminary results suggest that the herbal composition PHY-906 can be used as a modulator for CPT-11 chemotherapy to significantly improve and alleviate the toxic side effects of CPT-11 without compromising the anti-tumor efficacy of the CPT-11.

Example 6. Survival Rates of Colon 38 Inoculated Mice Treated with CPT-11 and PHY-906.

Mice were treated as set forth in Example 3 and their survival rates were evaluated over a twelve day period.

Mice inoculated with tumor cells were significantly less tolerable to the CPT-11 treatment than normal mice or mice treated with PHY-906 with or without CPT-11 treatment (Table 8). Thus, PHY-906 alleviates toxicity associated with CPT-11 treatment.

Table 8. Effect of PHY-906 on Survival Rate of Colon 38 Inoculated Mice Treated With CPT-11.

Treatment	Total Number	Number of Deaths	Survival Rate (%)
None	20	0	100
CPT-11, 400mg/kg	24	4	85.7
PHY-906, 1g/kg	15	0	100
CPT-11 + PHY906	24	0	100

Example 7. Effect of PHY-906 on Tumor Growth in Colon 38 Inoculated Mice Treated with L-OddC.

PHY-906 was evaluated as a modulator of L-OddC (beta-L-Dioxolane-cytidine) therapy for tumor growth in mice inoculated with Colon 38 tumor cells. Mice were subjected to a subcutaneous injection of murine Colon 38 tumor cells. Seven days after inoculation of the cancer cells, mice were treated with L-OddC (25 mg/kg) intra-peritoneally and oral administration of PHY-906 (1 g/kg, b.i.d.). The animals were then administered only with the same dose of PHY-906 continuously for the rest of the experiment.

As shown in Figure 4, treatment with L-OddC demonstrates that PHY-906 neither impedes nor impairs the antitumor efficacy of the L-OddC. In fact, the data suggest that this herbal medicine may actually enhance L-OddC anti-tumor activity.

Thus, these results suggest that the herbal PHY-906 can be used as a modulator for L-OddC chemotherapy to significantly improve and alleviate the toxic side effects without compromising the anti-tumor efficacy of the L-OddC.

Example 8. Effect of PHY-906 on Tumor Growth in Colon 38 Inoculated Mice Treated with VP-16.

PHY-906 was evaluated as a modulator of VP-16 (etoposide, a topoisomerase II inhibitor) therapy for tumor growth in mice inoculated with Colon 38 tumor cells. Mice were subjected to a subcutaneous injection of murine Colon 38 tumor cells. Seven days after inoculation with the cancer cells, mice were treated with VP-16 (25 mg/kg) intra-peritoneally and oral administration of PHY-906 (1 g/kg, b.i.d.). The animals were then administered only with the same dose of PHY-906 continuously for the rest of the experiment.

As shown in Figure 5, treatment with VP-16 demonstrates that PHY-906 neither impedes nor impairs the antitumor efficacy of the VP-16. In fact, the data suggest that this herbal medicine may actually enhance VP-16 anti-tumor activity.

Thus, these results suggest that the herbal PHY-906 can be used as a modulator for VP-16 chemotherapy to significantly improve and alleviate the toxic side effects without compromising the anti-tumor efficacy of the VP-16.

Example 9. Effect of PHY-906 on Tumor Growth in Colon 38 Inoculated Mice Treated with 5-fluorouracil.

PHY-906 was evaluated as a modulator of 5-fluorouracil (5-Fu) therapy for tumor growth in mice inoculated with Colon 38 tumor cells. Mice were subjected to a subcutaneous injection of murine Colon 38 tumor cells. Seven days after inoculation of the cancer cells, mice were

treated with 5-fluorouracil at two doses (250 mg/kg, one dose on day 0, or 30 mg/kg daily dose given from day 0 to day 4) intra-peritoneally and oral administration of PHY-906 (1 g/kg, b.i.d.). The animals were then administered only with the same dose of PHY-906 continuously for the rest of the experiment.

As shown in Figures 6 and 7, treatment with 5-fluorouracil demonstrates that PHY-906 neither impedes nor impairs the antitumor efficacy of the 5-fluorouracil. In fact, the data suggest that this herbal medicine may actually enhance 5-fluorouracil anti-tumor activity.

Thus, these results suggest that the herbal PHY-906 can be used as a modulator for 5-fluorouracil chemotherapy to significantly improve and alleviate the toxic side effects without compromising the anti-tumor efficacy of the 5-fluorouracil.

Example 10. Effect of PHY-906 on Tumor Growth in Colon 38 Inoculated Mice Treated with CPT-11 and Loperamide.

PHY-906 was evaluated as a modulator of CPT-11 therapy for tumor growth in mice inoculated with Colon 38 tumor cells in the presence of antidiarrhia medication Loperamide. Mice were subjected to a subcutaneous injection of murine Colon 38 tumor cells. Seven days after inoculation of the cancer cells, mice were treated with CPT-11 (400 mg/kg, i.p.), alone, in the presence of orally administered of PHY-906 (1 g/kg, b.i.d.) or in the presence of Loperamide (2 mg/kg, p.o., b.i.d.).

As shown in Figure 8, comparisons between PHY-906 and Loperamide demonstrate that CPT-11 in the presence of PHY-906 is more effective at reducing tumor growth (as determined as a percentage of initial tumor weight) than Loperamide.

These preliminary results suggest that the herbal PHY-906 is more effective than standard administration of Loperamide for delayed CPT-11 induced diarrhea.

Example 11. To determine the minimal effective dose (MED) and the optimal duration of PHY-906 administration when given in combination with irinotecan.

Introduction. Several studies indicate that Kampo medicine, which consists of seven herbs, is effective in preventing the occurrence of CPT-11-induced diarrhea in animals and in reducing the severity of CPT-11-induced diarrhea *in vivo* (Mori, 1998).

PHY-906 has also been evaluated in an *in vivo* animal model and has been shown to reduce the severity of irinotecan-induced toxicity. Accordingly, based on a long historical experience (1500 years) demonstrating safety in humans, the promising pre-clinical activity of this compound in an animal model, and the potential activity noted for a related herbal compound in this setting, a study can be conducted to evaluate the effect of PHY-906 on the severity of chemotherapy-induced toxicities such as weight loss, diarrhea, overall performance status, quality of life, and on the anti-tumor activity of irinotecan or other drugs in patients with refractory advanced colorectal cancer.

This study includes patients with histologically confirmed, 5-FU-refractory, advanced colorectal cancer. Measurable or evaluable disease is not required. Patients with central nervous system (CNS) metastases are eligible provided the CNS disease has remained stable for at least 4 weeks following completion of surgery, chemotherapy, and/or radiation therapy.

Participants in the study will be ≥ 18 years of age and will have no significant underlying medical diseases. All patients will have a performance status of ECOG 0-2, a life expectancy of at least 3 months, and have given informed consent. Patients must have fully recovered from the effects of any prior surgery and have not received wide-field radiation or any chemotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) of entry onto this study. An ANC ≥ 1500/μl, platelet count ≥40 ml/min, and a total bilirubin ≤2.0 mg/dl is required for entry onto study.

Pretreatment Evaluation. Prior to the start of treatment, all patients will have a complete history, physical examination, and a determination of their performance status. The laboratory studies will include a complete blood count (CBC) with differential, a serum albumin,

electrolytes, glucose, blood urea nitrogen (BUN), creatinine, serum calcium and magnesium, liver function tests, prothrombin and partial thromboplastin time, and a urinalysis.

Treatment. Irinotecan will be reconstituted from a lyophilized powder into 2 ml of sterile water, diluted in 100 ml of D5W, and administered over 90 min at a dose of 125 mg/m^2. Irinotecan chemotherapy will be administered on a weekly schedule for 4 weeks with a 2 week rest period in the outpatient clinic at each of the participating hospitals.

PHY-906 will be taken on an empty stomach 30 min prior to meals. On chemotherapy treatment days, the first dose will be taken before the administration of irinotecan.

PHY-906 will be administered orally three times a day before each meal starting at an initial dose of 0.60 g. (total daily dose, 1.80 gm/day). The dose of PHY-906 that is presently being used by patients in the Orient is 7.2 gm/day, and to date, no adverse events have been observed. Thus, the dose that we propose to start out with in this trial is ¼th the usual dose of the herbal medicine. PHY-906 will be given for an entire 4-week course of chemotherapy along with irinotecan with a 2-week rest. A minimum of three patients will be treated at this initial dose level of PHY-906. If 0 of 3 patients experience dose-limiting toxicity(DLT), once the 3 patients have completed a full 6-week cycle, then the next higher dose will be used for the subsequent group of 3 patients. In all patients, pharmacokinetic studies will be performed 24 hr after the start of the first cycle of chemotherapy.

If 1 of 3 patients experience DLT, then 3 more patients will be treated at the same dose level. If $\leq$1 of the next 3 experience DLT (1 or 2 of 6 total patients), the dose will be escalated to the next dose level except when those events occur during the doubling scheme, when the next escalation will be to level n + 1 on the modified Fibonacci scheme (Table 9).

Table 9. Dose Escalation Schedule of PHY-906.

Dose Level	Escalation	Total Dose (gm/day)
1	Starting	1.8
2	2 x level 1	3.6
3	2 x level 2	7.2
Once the 7.2 gm/day dose level is reached and no ≥ grade 2 toxicity is observed at this level "n", a modified Fibonacci escalation as shown below will be performed.		
N + 1	1.5 x level n	
N + 2	1.33 x level n + 1	
N + 3	1.25 x level n + 2	
All subsequent levels: 25% increments until the maximum tolerated dose is reached.		

Dose Escalation Schedule of PHY-906. If clinically indicated and considered necessary by the Principal Investigators, a lower dose level, rather than the level specified above, may be utilized.

The rate of subject entry and escalation to the next dose regimen will depend upon assessment of the safety profile of patients entered at each dose level. Toxicity will be evaluated and graded according to the NCI CTG Expanded Common Toxicity Criteria.

The antiemetic schedule for this protocol will consist of 1-2 mg of granisetron admixed in 50 ml normal saline and administered via ½ hr prior to chemotherapy on each treatment day. The antiemetics (administered intravenously or orally) will be repeated every 8 hr as needed to control nausea and/or vomiting. Treatment will be repeated every week for 4 consecutive weeks followed by a 2-week rest. This will constitute one cycle of therapy.

Diarrhea that occurs during or shortly after irinotecan infusion will be treated with atropine 0.5-1 mg IV. For diarrhea occurring ≥ 12 hr after irinotecan administration, patients will be treated with loperamide 4 mg orally at the first sign of diarrhea followed by 2 mg orally every 2 hr (4 mg orally every 4 hr at night) until there is complete resolution of the diarrhea for

at least 12 hr. If the diarrhea is bloody, associated with fevers $\geq$101.6° F, and continues unabated for $\geq$12 hr, the patient will be admitted tot he hospital for further evaluation and treatment.

Dose Modification of Irinotecan. There will be no dose escalation of irinotecan in this study. Dose modification for toxicity will be made as recommended in the package insert provided by the manufacture (Table 10).

Table 10. Recommended Dose Modifications for the Weekly and Once-Every 3-Week Schedule.

A new course of therapy should not begin until the granulocyte count has recovered to =1500/mm^3, and the platelet count has recovered to = 100,00/mm^3 , and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2 week delay, consideration should be given to discontinuing CAMPTOSAR.

Weekly Toxicity NCI Grade [b] Value	During a Course of Therapy	At the Start of the Next Courses of Therapy (After Adequate Recovery), Compared with the Starting Dose in the Previous Course [a]	
	Weekly	Weekly	Once every 3 weeks
No toxicity	Maintain dose level	*↑25mg/m² up to a maxi-mum dose of 150 mg/m2*	*↑ Maintain dose level*
Neutropenia 1 (1500 to 1999/mm³) 2 (1000 to 1499/mm³) 3 (550 to 999/mm³) 4 (<500/mm³	 Maintain dose level ↓25 mg/m² Omit dose, then ↓25mg/m²when resolved to ≤ grade 2 Omit dose, then ↓50mg/m²when resolved to ≤ grade 2	 *Maintain dose level* *Maintain dose level* *↓25 mg/m²* *↓50 mg/m²*	 *Maintain dose level* *Maintain dose level* *↓25 mg/m²* *↓50 mg/m²*
Neutropenic fever (grade 4 neutropenia & ≥ grade 2 fever)	Omit dose, then ↓ 50 mg/m² when resolved	*↓ 50 mg/m²*	*↓ 50 mg/m²*
Other hematologic Toxicities	Dose modifications for leukopenia, thrombocytopenia, and also based on NCI toxicity criteria and are the same at the start of subsequent courses of therapy are recommended for neuropenia above.		
Diarrhea 1 (2-3 stools/day > pretx [c]) 2 (4-6 stools/day > pretx [c]) 3 (7-9 stools/day > pretx [c]) 4 (= 10 stools/day >pretx [c])	 Maintain dose level ↓ 25mg/m² Omit dose, then ↓25 mg/m² when resolved to ≤ grade 2 Omit dose, then ↓50 mg/m² when resolved to ≤ grade 2	 Maintain dose level Maintain dose level ↓ 25 mg/m² ↓ 50mg/m²	 Maintain dose level Maintain dose level ↓ 50 mg/m² ↓ 50mg/m²
Other nonhemalogic Toxicities 1 2 3 4		 Maintain dose level Maintain dose level ↓25 mg/m² ↓50 mg/m²	 Maintain dose level Maintain dose level ↓25 mg/m² ↓50 mg/m²

a All dose modifications should be based on the worst preceding toxicity b National Cancer Institute Common Toxicity Criteria
c Pretreatment

<u>Response and Toxicity Assessment.</u> Toxicity will be assessed by weekly physical examination and blood counts and graded according to National Cancer Institute Common Toxicity Criteria. These evaluations and a complete chemistry profile will be repeated before each treatment.

Patients will also keep a daily record of their bowel habit and their use of anti-motility agents. This diary will include the time of ingestion of PHY-906, a recording of the frequency

and consistency of their bowel movements (formed, loose, or watery), and the anti-motility treatment which was used by the patient to manage this symptom.

A research nurse will contact each patient at least one time per week between visits during the first cycle to reinforce instructions on the management of diarrhea and the completion of the diary. Overall quality of life including asthenia, nausea, vomiting, loss of appetite will also be evaluated using established FAST methodology.

A pill count will be made by a pharmacist to each clinical visit for treatment to assess compliance with PHY-906. An evaluation of disease response will be made after every two treatment cycles. Response will be defined according to ECOG criteria and will be assessed in all patients with measurable or evaluable disease but will not constitute an endpoint in this study.

Pharmacokinetics of Irinotecan. In selected patients, pharmacokinetic studies will be performed to assess whether PHY-906 affects the metabolism and elimination of irinotecan. In these patients, the first dose of irinotecan will be given alone (cycle 1/day 1) and the PHY-906 will begin on day 2.

Blood samples will be collected in heparinized tubes immediately before irinotecan administration, 30, 60, 90 min during the infusion of irinotecan and 0.5, 1.5, 3.5 and 6 h after the end of the infusion on cycle 1, day 1, and on cycle 1 day 8. Samples will be immediately processed with 2.50 μl of plasma added to 500 μl of internal standard solution in polystyrene tubes. The internal standard solution will consist of camptothecin 50 μg/ml in acetonitrile acidified with glacial acetic acid, 4.0 ml in 100 ml. The samples will be vortexed for 30 sec, placed into a 40 °C water bath for 15 min, cooled at room temperature and then mixed with 900 μl of a 25 mM triethylamine buffer (pH 4.2). The supernatant will be transferred to 1.5 ml Eppendorf tubes, centrifuged for 4 min at 13,000 x g in a microcentrifuge, and an aliquot of the clear supernatant is analyzed by high performance liquid chromatography (HPLC).

Chromatographic analysis will be conducted on a Microsorh C18 (4.5 x 250 mm, 5μm particle size) reverse phase HPLC column eluted with 72:28 (v/v) 25 mM TEA/acetonitrile

buffer at 1ml/min utilizing a fluorescence detector with λEX 372 nm and λEm 535nm (Pharmacia & Upjohn SOP #UPJ-120-5). Maximum plasma concentration, terminal half-life, and AUC will be determined by non-compartmental analysis of the data utilizing PC-NONLIN software (Scientific Consulting Lexington, KY) and standard pharmacokinetic equations. The Pharmacokinetic studies will be performed on, cycle 1/day 1 and cycle 1/day 8, to determine whether prolonged exposure to PHY-906 produces a cumulative effect on the plasma clearance of irinotecan.

It should be understood that the foregoing discussion and examples present merely present a detailed description of certain preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. All articles, patents and patent applications that are identified above are incorporated by reference in their entirety.

The foregoing detailed description has been given for clearness of understanding only and no unnecessary limitations should be understood therefrom as modifications will be obvious to those skilled in the art.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

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